ARCHIVOS DE LA SOCIEDAD ESPAÑOLA DE OFTALMOLOGÍA

www.elsevier.es/oftalmologia

Original article

Streptozotocin-induced diabetes, and the optic nerve blood barrier☆

R. Alemán, B. Mompeó, I. Castaño*

Departamento de Morfología, Universidad de Las Palmas de Gran Canaria, Las Palmas, Spain

ARTICLE INFO

Article history:
Received 5 December 2015
Accepted 26 December 2015
Available online 28 March 2016

Keywords:
Optic nerve
Diabetes
Hyperglycaemia
Patency
Vessels

ABSTRACT

Objective: To study the features of the endoneurial micro-vessels of the optic nerve in streptozotocin-induced diabetic animals.
Methods: Optic nerves from control and streptozotocin-induced diabetic animals were studied by light and transmission electron microscopy. Patency was determined by indirect immunofluorescence albumin detection. The expression of major histocompatibility complex class II molecules was performed by direct immunofluorescence. The endoneurial vessels were counted, and the endothelial cell, the basement membrane, and the surface of the transverse section of the nerve were measured.
Results: Vessels of diabetic rats showed vessel wall thickening, preservation of pericytes, an increase in endothelial cell transcytosis, and an increased number of perivascular macrophage cells. It may be concluded that the effects of hyperglycaemia on the inner vessels of the optic nerve are more similar to the cerebral diabetic vessels than to the retinal vessels in diabetic animals.
© 2016 Sociedad Española de Oftalmología. Published by Elsevier España, S.L.U. All rights reserved.

Diabetes inducida por estreptozotocina y comportamiento de la barrera sanguínea del nervio óptico

Palabras clave:
Nervio óptico
Diabetes
Hiperglucemia
Permeabilidad
Vasos

RESUMEN

Objetivos: Conocer las características de la barrera sanguínea del nervio óptico de animales con diabetes inducida por estreptozotocina.
Método: Los nervios ópticos de animales diabéticos y controles se estudiaron mediante microscopia óptica y microscopia electrónica de transmisión. La permeabilidad de los vasos fue determinada mediante la detección de albúmina con inmunofluorescencia indirecta y la expresión de las moléculas del complejo mayor de histocompatibilidad clase II mediante inmunofluorescencia directa. Asimismo, se realizó un análisis morfométrico de la superficie del nervio, el número de vasos y el engrosamiento de la célula endotelial y lámina basal.

☆ Please cite this article as: Alemán R, Mompeó B, Castaño I. Diabetes inducida por estreptozotocina y comportamiento de la barrera sanguínea del nervio óptico. Arch Soc Esp Oftalmol. 2016;91:170–176.
* *Corresponding author.*
E-mail address: raleman@dmor.ulpgc.es (R. Alemán).
2173-5794/© 2016 Sociedad Española de Oftalmología. Published by Elsevier España, S.L.U. All rights reserved.

Resultados: Los microvasos de los nervios ópticos de los animales diabéticos por efecto de la estreptozotocina se caracterizaron por un incremento en el grosor de su pared, conservación de los pericitos, incremento de la transcitosis en la célula endotelial y la presencia de una población importante de macrófagos perivasculares. En general, las manifestaciones del efecto de la hiperglucemia en el nervio óptico fueron más semejantes a las descritas para la microcirculación cerebral que a las descritas para la retina.

© 2016 Sociedad Española de Oftalmología. Publicado por Elsevier España, S.L.U. Todos los derechos reservados.

Introduction

Diabetic optic neuropathy is a clinical condition involving the optic pathway of diabetic patients. It can course asymptomatically, appearing only in neurophysiological studies, and it can also appear without the presence of diabetic retinopathy. Although the etiopathogenic mechanism of said neuropathy is not well established, many studies indicate vascular origin. Anterior optic neuropathy involves small choroidal circulation vessels that feed the anterior portion of the optic nerve before reaching the *lamina cribrosa*, whereas posterior optic neuropathy, which seems to be more frequent in diabetic patients,[1] could mainly involve microvessels reaching the nerve from the pial circulation network.

Taking into account the above and even though the vascular origin of diabetic neuropathy in any organ is broadly accepted,[2] the structural changes of vessels in this disease are largely unknown. On the other hand, the demonstrated production of inflammatory cytokines due to the oxidative stress taking place in diabetes[3] has determined the possible therapeutic usefulness of steroids and nonsteroid anti-inflammatories for treating diabetic neuropathy.

In a previous article[4] the authors demonstrated morphological alterations in optic nerve fibers in diabetes-induced rats by means of streptozotocin. Said alterations included damage to, and the disappearance of, large size nerve fibers and the presence of myelin remains inside the nerve. However, the authors have not found studies relating the alterations found in the optic nerve fibers with vascular origin.

The objective of this paper was to study the morphological, structural, morphometric and functional changes of optic nerve blood vessels and the blood barrier in an experimental diabetes model, to determine whether modifications took place in the microvessels of hyper-glycemic animals that could explain the alterations found in optic nerve fibers. In the case of finding said alterations, comparing said changes with those described in the microvessels of the retina and brain as a consequence of hyperglycemia.

Materials and methods

Animals and treatment of samples

Hyperglycemia was induced in a group of two-month old Sprague Dawley rats with a weight of 263 ± 58.65 g by means of a single intraperitoneal injection of streptozotocin (65 mg/kg) in citrate tamponade with a pH of 4.5. A group of control animals with the same characteristics was submitted to a single tamponade injection. The level of glycemia reached by diabetic animals exceeded 350 mg/dl, whereas in control animals said level was under 110 mg/dl. The diabetic animals were not administered insulin for correcting hyperglycemia.

The glucose levels in plasma were assessed weekly by means of an oxidase glucose reaction system (Menarine Glucocard test, Glucocard International, Florence, Italy).

After 6 and 12 weeks, 18 diabetic animals and 18 controls were sacrificed after being anesthesized with an intraperitoneal injection of equithesin 3.3 ml/kg (9.5 mg pentobarbital sodium, 76 ml ethanol, 42.5 mg chloral hydrate, 428 mg propylene glycol, 21 mg magnesium sulfate, and distilled water up to 1 l).

The circulation system was washed by means of cardiac perfusion with physiological saline solution in order to subsequently fix the animal. Two types of fixatives were utilized: 2% glutaraldehyde solution in 0.1 M, pH 7.4 phosphate tamponade, and 4% paraformaldehyde solution, depending on whether the samples were allocated for ultrastructural study or immunofluorescence techniques, respectively. The optic nerves were obtained through craniotomy and preserved in the same fixative utilized for perfusion. The experiment was carried out following the guidelines of RD 1201/2005 dated October 10 (Spain) on the protection of animals utilized for research and other scientific purposes.

The optic nerves fixated with glutaraldehyde were post-fixed in OsO_4 1%, dehydrated in ethanol and included in agar resin 100 by means of a routine technique. One-half micron sections were stained with toluidine blue in borax and ultrafine 500 Å sections with uranyl acetate and lead citrate. The nerves fixated in paraformaldehyde were included in paraffin molds.

Morphometry

The morphometric studies of optic nerve vessels were carried out utilizing both types of sections. The analysis was made over ultrafine section photographs with an overall magnification of 7200×, obtained with a Philiphs 301 electronic transmission microscope (Royal Philips Electronics, Amsterdam, Holland).

The intraneural vessels were selected randomly, measuring basement membrane, endothelium area, vascular perimeter and gauge of each vessel.

Download English Version:

https://daneshyari.com/en/article/4007988

Download Persian Version:

https://daneshyari.com/article/4007988

Daneshyari.com